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Indian Standard

PHOSPHATE SOLUBILISING BACTERIAL INOCULANT (PSBI) — SPECIFICATION

ICS 65.0.80

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

October 2000

Price Group 2

ANNEX C

AMENDMENT NO. 1 JUNE 2008
TO
IS 14807 : 2000 PHOSPHATE SOLUBILISING BACTERIAL INOCULANT (PSBI) — SPECIFICATION

(*Page* 1, *clause* **4.1**, *line* 2) — Substitute '10^8' *for* '10^7'.

(*Page* 1, *clause* **4.2**, *line* 6) — Substitute '150 to 212 µ (72 to 100 mesh) IS sieve' *for* '100 µ sieve'.

(FAD 7)

Reprography Unit, BIS, New Delhi, India

AMENDMENT NO. 2 MAY 2012
TO
IS 14807 : 2000 PHOSPHATE SOLUBILISING BACTERIAL INOCULANT (PSBI) — SPECIFICATION

[*Page* 1, *clause* **6.1**(h)] — Substitute 'Net quantity in kg and area meant for;' *for* 'Net mass in kg and area meant for;'.

(FAD 7)

Reprography Unit, BIS, New Delhi, India

Soil Quality and Improvement Sectional Committee, FAD 27

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Soil Quality and Improvement Sectional Committee had been approved by the Food and Agriculture Division Council.

The phosphate solubilising bacterial inoculants (PSBI) include different groups of soil micro-organisms which convert insoluble phosphatic compounds into soluble form. The species of *Pseudomonas, Micrococcus, Bacillus, Flavobacterium* are active in bioconversion. The most efficient bacterial isolates are identified as *Pseudomonas striata, Pseudomonas rathonis, Bacillus polymyxa*. These efficient micro-organisms have shown capability to solubilise phosphorus content supplied through fertilizer application in the soil. Many brands of PSBI inoculants are marketed in the country and they have been found to vary in quality. Hence, the need was felt for Indian Standard not only to test the quality of inoculants in order to provide the farmers with certified inoculants but also to producers improve quality of their products.

It is obligatory on the part of the manufacturers to employ qualified Soil Microbiologist/Microbiologist/ Agricultural Graduates/Graduates in Biology trained in soil microbiology in their staff. Manufacturers shall also maintain a quality control laboratory capable of carrying out the tests according to this standard.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

PHOSPHATE SOLUBILISING BACTERIAL INOCULANT (PSBI) — SPECIFICATION

1 SCOPE

This standard prescribes the requirements, method of sampling and tests for Phosphate Solubilising Bacterial inoculants.

2 REFERENCE

The following Indian Standard contains provisions which, through reference in this text, constitutes provisions of this standard. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below:

IS No.	*Title*
8268 : 1986	Specification or Rhizobium inoculant (*first revision*)

3 TERMINOLOGY

3.1 For the purpose of this standard the following definitions shall apply.

3.1.1 *Phosphate Solubilising Bacterial Inoculants (PSBI)*

PSBI is a product having high population of a strain(s) of bacteria intended to solubilise insoluble phosphate of the soil.

3.1.2 *Group of Crops*

PSBI is beneficial for all types of crops in terms of solubilisation of fixed phosphate of soil.

4 REQUIREMENTS

4.1 When tested by the method prescribed in Annex A of the standard, PSBI shall contain 10^7 viable Phosphate Solubilising Bacterial cells/g of the carrier material on dry mass basis.

4.2 PSBI shall be carrier based, colour depending on the colour of the carrier. Carrier material such as peat, lignite, charcoal or similar material may be used. It shall be neutralized with calcium carbonate and then sterilized. When tested it shall pass through 100 μ sieve.

4.3 PSBI when tested by the method prescribed in Annex A, shall have no contamination with other micro-organisms at 10^5 dilution.

4.4 When tested by the method prescribed in Annex B of IS 8268, the *p*H of PSBI shall be 6.5 to 7.5.

4.5 When tested by the method prescribed in Annex C, PSBI shall have *phosphate solubilising* capacity in the range of minimum 30 percent in terms of zone formation minimum 10 mm solubilisation zone in a prescribed solid medium having at least 3 mm thickness.

4.6 Specified mother culture be obtained from any recognized institution maintaining the mother culture. The manufacturer may control the quality of broth as given in Annex D.

NOTE — At present National Biofertilizer Development Centre (NBDC), Ghaziabad and its six Regional Centres located at Bangalore, Bhubaneshwar, Hissar, Imphal, Jabalpur and Nagpur; Indian Agricultural Research Institute (IARI), New Delhi; Tamil Nadu Agricultural University (TNAU), Coimbatore; University of Agricultural Science, Bangalore are sources for supplying the mother culture.

5 PACKING

PSBI shall be packed in polyethylene packs, thickness of which shall not be less than 100 (micron).

6 MARKING

6.1 Each polyethylene pack shall be marked legibly and indelibly with the following information:

a) Name of the product, specially as Phosphate Solubilising Bacterial inoculant;
b) Name and address of the manufacturer;
c) Crop(s) for which intended;
d) Type of the carrier used;
e) Batch number;
f) Date of manufacture;
g) Expiry date which shall not be less than 6 months from the date of manufacture;
h) Net mass in kg and area meant for;
j) Storage instructions worded as under : "STORE IN COOL PLACE AWAY FROM DIRECT SUNLIGHT AND HEAT"; and
k) Any other information required under the *Standards of Weights and Measures (Packaged Commodities) Rule,* 1977.

6.2 Items (c), (f) and (g) shall be printed on a coloured ink background

6.3 Direction for use of PSBI shall be printed briefly on the packets as given in Annex E of the standard. A separate pamphlet may preferably be given with it.

6.4 BIS Certification Marking

The product may also be marked with the Standard Mark.

6.4.1 The use of the Standard Mark is governed by the provisions of *Bureau of Indian Standards Act*, 1986 and Rules and Regulations made thereunder. The details of conditions under which the licence for the use of Standard Mark may be granted to manufacturer or producers may be obtained from the Bureau of Indian Standards.

6.5 Storage

PSBI shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at a temperature of 20°C and not exceeding 30°C. It shall also be the duty of the manufacturer to instruct the retailers and, in turn, the users about the precautions to be taken during storage.

7 SAMPLING

The representative samples of the PSBI shall be drawn as prescribed in Annex F of IS 8268.

ANNEX A

(*Clauses* 4.1 *and* 4.3)

DETERMINATION OF NUMBER OF PHOSPHATE SOLUBILISING BACTERIAL CELLS

A-1 APPARATUS

A-1.1 Pipettes — Graduated, 1 ml and 10 ml.

A-1.2 Conical Flasks — 150 ml and 250 ml.

A-1.3 Screw capped tubes of 10 ml capacity.

A-1.4 Incubator

A-1.5 Petri Dishes

A-1.6 Hot Air Oven

A-1.7 Autoclave

A-1.8 *p*H meter

A-2 REAGENTS

A-2.1 Use a medium of the following composition:

Glucose	10.0 g
Tri-calcium phosphate	5.0 g
Ammonium sulphate	0.5 g
Magnesium sulphate	0.1 g
Sodium chloride	0.2 g
Yeast extract	0.5 g
Manganese sulphate	Trace
Ferrous sulphate	Trace
Distilled water	1 000 ml
Agar	15.0 g
*p*H adjusted to 7 ± 0.2	

A-2.3 Sterilising and preparation process for plates is the same as described in IS 8268.

A-3 PREPARATION OF SERIAL DILUTION FOR CELL COUNT METHOD

Dispense 30 g of PSBI in 270 ml of sterile water and shake for 10 minutes on a reciprocal shaker. Make serial dilutions up to 10^7 level. Pipette out 0.2 ml aliquots of 10^5 to 10^7 dilution and deliver it on the petri dishes containing set medium as described in **A-2.1**. Spread the aliquots over the plate. Invert the plates and place them in the incubator at 28 ± 2°C for 3 days. Use 3 replicates of 10^5, 10^6 and 10^7 dilution.

A-4 COUNTING

Count the total number of colonies on the plates including colonies with solubilization zone with the help of a colony counter.

A-5 METHODS FOR COUNTING SOLUBILIZATION ZONES

a) Take 10 g of PSBI (BF) in 90 ml in water.

b) Make a ten fold dilution series up to 10^7.

c) Take 0.2 ml aliquots of 10^5 to 10^7 dilution using sterile pipettes and delivered to petri dishes containing Pikowskeyi media.

d) Spread it uniformly. Invert the plates and incubate them up to 2 weeks at 28° ± 2°C.

e) Count the colonies showing hallow cones and measure their diameter. Minimum acceptable zone is 10 mm in diameter.

ANNEX B

(*Clause* 4.4)

DETERMINATION OF *p*H AND MOISTURE PERCENTAGE

B-1 *p*H

Make suspension of 20 g of the PSBI into 50 ml of distilled water and shake on a rotary shaker for 2 h. Filter this suspension and determine the *p*H of the filtrate with the help of *p*H meter.

B-2 DETERMINATION OF MOISTURE OF BIOFERTILIZER PACKETS (METHOD)

Heat 10 g of sample for 12-16 hours in an air oven at 100-105°C. Cool in a desiccator and weigh. The loss in weight represents the moisture. Calculate the moisture percentage on air dry weight basis, by multiplying the loss in weight by ten.

ANNEX C

(*Clause* 4.5)

DETERMINATION OF SOLUBLE PHOSPHORUS USING ASCORBIC ACID

C-0 PRINCIPLE

Soluble phosphorus forms hetropoly molybdophosphate complex with molybdate ions which on reduction produces a characteristic blue colour measured at 840 to 880 nm.

Considering the higher stability of the ascorbic acid, easiness to handle, higher tolerance to the concentration of interfering ions, possibilities to use it with all types of acids and higher stability of the developed colour (from 10 to 60 min), ascorbic acid instead of stanous chloride is now-a-days used as the reducing agent for the hetropoly molybdophosphate complex formed by the soluble phosphate ions on addition of ammonium molybdate solution.

C-1 APPARATUS

Spectrophotometer capable of transmission measurements at 840 to 880 nm.

Extractant : It is olsen extract.

C-2 REAGENTS

C-2.1 Ammonium Molybdate $[(NH_4)_6 MO_7O_{24}.4H_2O]$

C-2.2 *L*-Ascorbic Acid

C-2.3 *p*-Nitrophenol

C-2.4 $4NH_2SO_4$

C-3 PREPARATION OF REAGENTS

C-3.1 Sulphomolybdic Acid

C-3.1.1 Take 20 g of ammonium molybdate and dissolve in 300 ml of distilled water.

Add slowly 450 ml of 10 N H_2SO_4.

Cool the above mixture and add 100 ml of 0.5 percent solution of antimony potassium tartrate.

Cool and make the volume to one litre. Store in glass bottle away from direct sunlight.

C-3.2 Preparation of Mixed Reagent

Add 1.5 g of *L*-ascorbic acid in 100 ml of the above stock solution and mix. Add 5 ml of this solution to develop colour. Mixed reagent is to be prepared fresh as it does not keep for more than 24 h.

C-3.3 Procedure

C-3.3.1 Weigh the required material in a 100 ml conical flask.

C-3.3.2 Add 50 ml of extractant and shake it for 30 min on a rotary shaker.

C-3.3.3 Filter the suspension through Whatman filter paper No. 40. If the filtrate is coloured then add a tea spoon of Darco-60 (activated phosphorus free carbon), reshake and filter.

C-3.3.4 Take a known aliquot (5 to 25 ml) of the extract in a 50 ml volumetric flask.

C-3.3.5 Add 5 drops of *p*-nitrophenol indicator (1.5 percent solution in water) and adjust the *p*H of the extract between 2 and 3 with the help of $4NH_2SO_4$. The yellow colour will disappear when the *p*H of the solution becomes 3. Swirl gently to avoid loss of the solution along with the evolution of CO_4.

C-3.3.6 When the CO_2 evolution has subsided, wash down the neck of the flask and dilute the solution to about 40 ml.

C-3.3.7 Add 5 ml of the sulphomolybdic acid mixed reagent containing ascorbic acid, swirl the content and make up the volume.

C-3.3.8 Measure the transmission after 30 min at 880 nm using red filter. The blue colour developed remains stable up to 60 minutes.

C-3.3.9 Record the concentration of Phosphorus (*P*) in the extract from the standard curve and calculate the concentration of soluble Phosphorus as follows:

C-4 CALCULATIONS

a) Weight of the substance taken = *x*g
b) Volume of the extractant added = 50 ml
c) Volume of the extract taken for *P* determination = *y* ml
d) Volume made after colour developed = 50 ml
e) Reading from the standard curve against percent transmission recorded = *z* ppm
f) Soluble Phosphorus,

$$\text{percent } P = \frac{z \times 50 \times 10^{-6} \times 50}{y \cdot x} \times 100$$

C-4.1 Preparation of Standard Curve

Prepare standard curve using 0.1 to 0.6 ppm *P* in 50 ml volumetric flask. Plot the standard curve by taking concentration of soluble *P* on *x*-axis and percent *T* on *y*-axis using a semi-log graph paper. It is a straight line relationship between the soluble *P* and percent *T* when plotted on a semi-log graph paper.

ANNEX D

(*Clause* 4.6)

GUIDELINES ON MAINTENANCE AND PREPERATION OF CULTURE AND QUALITY CONTROL AT BROTH STAGE

D-1 MAINTENANCE OF PURE CULTURES

D-1.1 Maintain pure culture of PSBI on the medium as described in **A-2.1** in the form of slants.

D-1.2 Transfer a loopful of pure culture to each of the agar slants aseptically in an inoculation room and incubate at 28 ± 2°C for three days. Always keep pure culture below 5°C.

D-2 PREPARATION OF INOCULUM CULTURE AND MASS CULTURE

Inoculum culture and mass culture shall be prepared as described in **D-2.1**, **D-2.2** and **D-3.1** of the IS 8268 changing the media composition as mentioned in **A-2.1** of this standard.

D-3 QUALITY CONTROL TEST RECOMMENDED AT BROTH STAGE

D-3.1 Qualitative Test

D-3.1.1 Check for free from visible contaminants by microscope and observing solubilization zones.

D-3.1.2 The *p*H of bacterial broth shall normally be between 6.5 to 7.

D-3.1.3 Gram staining test shall be carried out as described in **D-4.1.3.1** and **D-4.1.3.2** of IS 8268.

D-3.2 Quantitative Test

D-3.2.1 *Viable Cell Count*

ANNEX E

(*Clause* 6.3)

DIRECTION FOR USE OF PHOSPHATE SOLUBILISING BACTERIAL INOCULANTS

E-1 The contents of the packet are sufficient enough to the given area to be broadcast or given seedlings for root dipping depending on the specified crops as denoted on the packet. Mix the inoculants with seeds gently with the minimum amount of water, taking care to avoid damage to seed coat. dry the inoculated seed under shake over clean dry paper organic bag and sow them immediately.

E-2 In order to solubilize fixed soil phosphate use PSBI for all type of crops. Use before the expiry date and do not expose to direct sunlight or heat.

E-3 PSBI is not a chemical fertilizer hence do not mix inoculated seeds or PSBI with agro-chemicals.

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